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Ligand trapping in epithelial layers and cell cultures

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Abstract

We analyze a stochastic model that describes receptor-mediated ligand trapping in epithelial layers and cell culture assays. In both cases, the problem is reduced to diffusion of a Brownian particle between the partially absorbing and reflective surfaces. We derive an analytical expression for the spatial distribution of the trapping points and identify the domains of applicability of the two limiting regimes. We conclude that a thin layer approximation is applicable for ligand trapping in epithelial layers while a typical cell culture experiment is appropriately described within an infinite layer approximation.

Keywords: Ligand; Receptor; Diffusion; Cell culture; Epithelial layer

1. Introduction

A large fraction of cell communication in tissues is mediated by secreted chemical signals [1]. A secreted signal can be a small molecule, such as nitric oxide, or a peptide growth factor with several hundred aminoacids. In cell communication mediated by secreted growth factors, the cell can both frequently secrete the signal and bind it using a cognate cell surface receptor [2,3]. Many aspects of cell and tissue physiology depend on the spatial range of secreted molecules [4]. Recent experiments have identified a large number of receptor-mediated processes that can control the spatial range of diffusible signals [5–7]. Here, we analyze a stochastic model that describes receptor-mediated ligand trapping in two different experimental set-ups: an epithelial layer and a cell culture assay.

In epithelial layers, the cells are tightly adhering to each other and secreted ligands diffuse in the extracellular space between the epithelium and an adjacent surface. One of the simplest scenarios has been identified in *Drosophila* egg development, where ligands of the EGF receptor diffuse in a thin gap between the follicular epithelium and the surface of the oocyte [8,9]. Receptors are uniformly distributed on the surface of the follicle cells and are absent on the surface of the oocyte. Effectively, ligands diffuse between the partially absorbing and reflecting surfaces. The efficiency of trapping by the partially absorbing surface is a

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function of the rate constant κ that depends on the level of receptor expression (R_{tot}), the cell surface area (A) and the binding rate constant (k_{on}): $\kappa = R_{tot}k_{on}/A$. In developing epithelial layers, cell surface receptors play two roles: they both transduce and localize the secreted ligands. The latter process has been termed 'ligand trapping' [10].

In a typical cell culture assay, cells are randomly distributed on a substrate which is covered by a layer of liquid medium. The cells can both secrete diffusing ligands and capture them by cognate receptors. Thus, a ligand is diffusing in a layer between two boundaries, the upper boundary is reflecting and the lower boundary is a reflecting plane randomly covered by non-overlapping partially absorbing circular traps, which model the cells. A ligand secreted by a cell in a cell culture assay can be captured by the 'parent' cell or one of its neighbors [11,12]. Analysis of the fraction of the ligand recaptured by the ligand-producing cell has been presented elsewhere [13,14]. Here, we focus on the ligands that escape. The spatial range of trapping of these ligands is important in several applications. For example, a large number of experiments indicate that a radiation bystander effect (i.e. the ability of the radiation to affect the cells that were not in direct contact with radiation) is mediated by secreted chemical factors [15–17]. Analysis of the spatial extent of this effect requires the analysis of ligand trapping in cell culture assays.

Brownian dynamics simulations show that, over a wide range of the parameter values, the average trapping distance greatly exceeds both the cell size and the average distance between the neighboring cells on the surface [14]. This implies that one can (1) replace the inhomogeneous boundary condition on the cell-covered boundary by a homogeneous one with an effective trapping rate and (2) assume that all trajectories start from the same point, i.e. neglect their initial distribution over the surface of the signal-producing cell. The effective trapping rate constant is given by:

$$\kappa = \kappa_{cell}\sigma/(1 + \pi\kappa_{cell}r_{cell}/4D),$$

where κ_{cell} characterizes the trapping efficiency of the cell surface and is a function of the receptor expression level, the cell surface area and the binding rate constant, r_{cell} is the cell radius, σ is the fraction of the cell-covered boundary occupied by the cells, and D is the diffusion constant of a secreted ligand.

Thus, to characterize the ligand trapping in epithelial layers and in cell culture assays, one has to analyze the problem of ligand diffusion between the surfaces with the partially absorbing and reflecting boundary conditions. Here, we derive an expression for the distribution function of the trapping points in this problem and show how the distribution depends on the surface reaction rate constants, the ligand diffusivity and the height of the medium. Our general results are in the form of the infinite series expansions. We identify the domains of the applicability for the two limiting regimes, the thin-layer and the infinite layer approximations, where the general expressions significantly simplify.

2. Theory

Consider a ligand diffusing in a layer of thickness h between two planar boundaries located at $z=0$ and $z=h$. The upper boundary at $z=h$ is reflecting, while the lower boundary at $z=0$ is partially absorbing. The ligand starts from the origin and eventually is trapped somewhere on the lower boundary. We wish to determine the probability density of the trapping points $p_h(r)$, where r is the distance from the origin to the trapping point. By definition of the probability density, $p_h(r)\mathrm{d}r$ is the probability for the ligand to be trapped in a ring between r and $r+\mathrm{d}r$. Having in hand the result for $p_h(r)$ and the number of ligands released at different points one can easily calculated the number of ligands trapped at any point of the partially absorbing boundary.

To find $p_h(r)$ one has to solve the diffusion equation in the layer with reflecting and partially absorbing (radiation) boundary conditions at $z=h$ and $z=0$, respectively. The solution is used to find the distribution of the trapping points for trajectories trapped between t and $t+\mathrm{d}t$. Integrating this distribution with respect to time with the weight factor given by the probability to be trapped

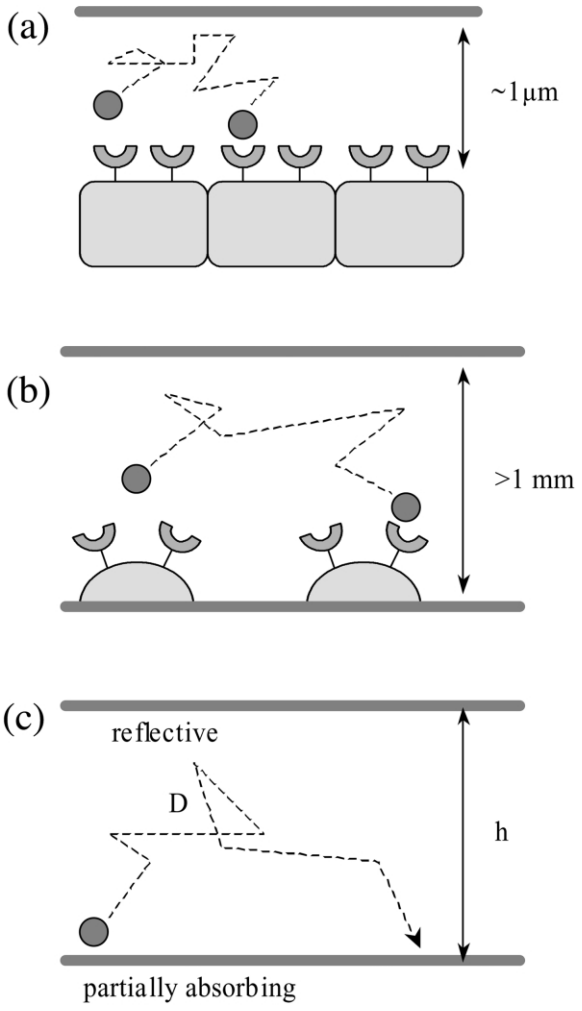

Fig. 1. (A) Ligand trapping in epithelial layers. A typical height of the layer for ligand diffusion is ~1 μm. (B) Ligand trapping in a cell culture assay. A typical height of the layer is >1 mm. (C) Both experimental set-ups lead to the problem where a Brownian particle is diffusing between the surfaces with the partially absorbing and reflective boundary conditions.

between t and $t+\mathrm{d}t$ one finds the desirable density $p_h(r)$ (Fig. 1).

Diffusion in the lateral and z directions occur independently. As a consequence, the ligand propagator (or the Green's function) is a product of the corresponding propagators: the lateral propagator, which is the free propagator in two dimensions, $G_{2\mathrm{d}}(r,\,t)=(4\pi Dt)^{-1}\exp(-r^2/4Dt)$, where D is the ligand diffusion constant, and the propagator $g(z,\,t)$, which is discussed in Appendix A. The probability density for the ligand lifetime, $\varphi_h(t)$, is given by $\varphi_h(t)=\kappa g(0,\,t)$. By definition, $\varphi_h(t)\mathrm{d}t$ is the probability that the ligand is trapped between t and $t+\mathrm{d}t$. For trajectories trapped between t and $t+\mathrm{d}t$ the trapping point density is

$$2\pi r G_{2\mathrm{d}}(r,\,t)=\frac{r}{2Dt}\exp\left(-\frac{r^2}{4Dt}\right) \tag{1}$$

Using this density we can write $p_h(r)$ as

$$\begin{aligned} p_h(r) &= 2\pi r\int_0^\infty G_{2\mathrm{d}}(r)\varphi_h(t)\mathrm{d}t \\ &= \frac{r}{2D}\int_0^\infty \exp\left(-\frac{r^2}{4Dt}\right)\frac{\varphi_h(t)}{t}\mathrm{d}t \end{aligned} \tag{2}$$

In what follows we substitute an expression for $\varphi_h(t)$ derived in Appendix A into Eq. (2) and carry out the integration with respect to time.

One can find $\varphi_h(t)$ by solving a 1D problem of a particle diffusing in an interval of length h with reflecting and partially absorbing end points (see Appendix A for details):

$$\varphi_h(t)=2D^2\kappa\sum_{n=1}^{\infty}\frac{k_n^2}{D\kappa+h(\kappa^2+D^2k_n^2)}\exp(-k_n^2Dt) \tag{3}$$

where κ is the rate constant that characterizes the trapping efficiency of the partially absorbing boundary. The eigenvalues k_n are positive roots of the equation:

$$\frac{D}{\kappa}k_n=ctg(k_nh),\quad n=1,\,2,\ldots \tag{4}$$

Substituting $\varphi_h(t)$ in Eq. (3) into Eq. (2) and carrying out the integration one finds

$$p_h(r)=2D\kappa r\sum_{n=1}^{\infty}\frac{k_n^2}{D\kappa+h(\kappa^2+D^2k_n^2)}K_0(k_nr) \tag{5}$$

where $K_0(z)$ is a modified Bessel function of the second kind [18].

The expression in Eq. (5) is one of the main results of this communication. Now we use it to analyze the dependence of the trapping point density on the layer thickness, h. When $h \ll D/\kappa$, $p_h(r)$ in Eq. (5) simplifies and reduces to

$$p_h(r) \approx \frac{r\kappa}{Dh} K_0\left(\sqrt{\frac{\kappa}{Dh}}\, r\right) \tag{6}$$

This 'thin layer' asymptotic behavior of $p_h(r)$ has a simple interpretation. When $h \to 0$ the local density along the z-coordinate rapidly equilibrates. As a result, the problem reduces to one of a 2D diffusion with uniform trapping that is characterized by the rate constant κ/h. Solving this problem one arrives at $p_h(r)$ in Eq. (6). As $h \to 0$ the effective rate constant κ/h tends to infinity, and the ligand is instantly trapped at the starting point, i.e.

$$\lim_{h \to 0} p_h(r) = \delta(r) \tag{7}$$

At finite values of h, $p_h(r)$ is a bell-shaped function: initially it grows with r from 0 to a maximum value and then decreases and tends to 0 as $r \to \infty$. In the limiting case of unbounded diffusion in semi-infinite space, $h \to \infty$, $p_\infty(r)$ is a monotonically decreasing function of r. Here the sum in Eq. (5) can be replaced by the integral, and $p_h(r)$ takes the form

$$p_\infty(r) = \frac{2\kappa}{\pi D} \int_0^\infty \frac{x^2}{x^2 + (\kappa r/D)^2} K_0(x)\,\mathrm{d}x \tag{8}$$

In Fig. 2 we show $p_h(r)$ for several values of h. This figure illustrates variation of the density $p_h(r)$ due to the change in the layer thickness. We find that the expressions in Eqs. (6) and (8) are applicable for $h/D \ll 0.03$ and $hk/D > 1$, respectively.

Function $p_\infty(r)$ very slowly approaches 0 as $r \to \infty$. Its large-r asymptotic behavior is given by $p_\infty(r) \simeq D/(\kappa r^2)$, $r \to \infty$. As a consequence, all moments of $p_h(r)$ diverge as $h \to \infty$. The mth moment of the trapping distance at arbitrary h is given by

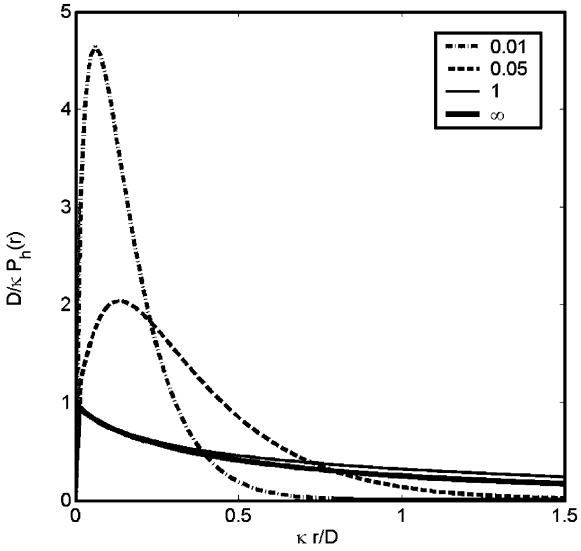

Fig. 2. Probability density function for the trapping distances evaluated at several values of dimensionless parameter $h\kappa/D$. The legend shows the curves corresponding to the different parameter values.

$$\langle r^m \rangle_h = \int_0^\infty r^m p_h(r)\,\mathrm{d}r = 2^{m+1}\left[\Gamma\left(\frac{m}{2}+1\right)\right]^2 D\kappa \times \sum_{n=1}^{\infty} \frac{1}{k_n^m\left[D\kappa + h(\kappa^2 + D^2 k_n^2)\right]} \tag{9}$$

To derive this expression we have used the relation in item 11.4.22 from Ref. [18]. One can check that, as $h \to 0$, $\langle r^m \rangle_h$ tends to 0 as $h^{m/2}$. The dependence of the average trapping distance, $\langle r \rangle_h$, on the layer thickness is shown in Fig. 3.

Another useful characteristic of the range of signaling is the fraction of trajectories, which are trapped at distances larger than r. We denote this fraction by $F_h(r)$

$$F_h(r) = \int_r^\infty p_h(r')\,\mathrm{d}r' = \int_0^\infty \exp\left(-\frac{r^2}{4Dt}\right)\varphi_h(t)\,\mathrm{d}t = 2D\kappa r \sum_{n=1}^{\infty} \frac{k_n}{D\kappa + h(\kappa^2 + D^2 k_h^2)} K_1(k_n r) \tag{10}$$

For narrow layers with $h \ll D/\kappa$, this fraction is given by

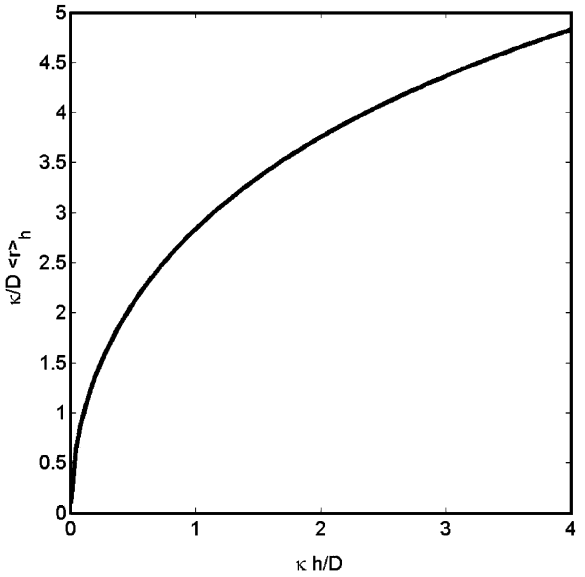

Fig. 3. Average trapping distance as a function of dimensionless parameter $h\kappa/D$.

$$F_h(r) = \sqrt{\frac{\kappa}{Dh}} r K_1\left(\sqrt{\frac{\kappa}{Dh}} r\right) \tag{11}$$

In the case of unbounded diffusion in semi-infinite space $F_h(r)$ takes the form

$$F_\infty(r) = \frac{2\kappa r}{\pi D}\int_0^\infty \frac{x}{x^2+(\kappa r/D)^2} K_1(x)\mathrm{d}x \tag{12}$$

The dependence $F_h(r)$ is shown in Fig. 4 for several values of $h\kappa/D$.

3. Concluding remarks

We have derived an expression for the distribution function of the trapping points for a Brownian particle diffusing between the partially absorbing and reflecting surfaces. This problem arises in the analysis of ligand transport in epithelial layers and cell culture assays. In both cases, it is important to determine how far does the ligand travel after it had been secreted. By analyzing the infinite series expansions of the distribution functions, we have identified the domains of applicability of the two limiting regimes. The infinite layer approximation is accurate for $h\kappa/D > 1$. The thin gap approximation is accurate for $h\kappa/D < 0.03$.

For developing epithelial layers the typical value of the parameters are: receptor expression $\sim 10^4$ receptors/cell, a cell area ~ 75 μm^2, a forward rate constant $\sim 10^8$/M min, ligand diffusivity $\sim 10^{-7}$ cm^2/s and the height of the layer ~ 1 μm. This leads to $h\kappa/D \approx 0.01$, which is within the regime of the applicability of the thin layer approximation. This estimate justifies the analysis of receptor-mediated epithelial patterning in Refs. [19,20], which is in fact based on the thin layer approximation. At the same time, the height of the medium in a typical cell culture experiment is 2–3 mm and the cell density is $\sim 50\%$ [11,12,21]. Using the same cellular parameters and the diffusivity in free solution $\sim 10^{-6}$ cm^2/s, we get $h\kappa/D > 1.5$. Hence, ligand trapping in a typical cell culture assay can be accurately described using the infinite layer approximation. Both the thin layer and infinite layer approximations are captured by simple formulas given in Eqs. (6) and (8). In the

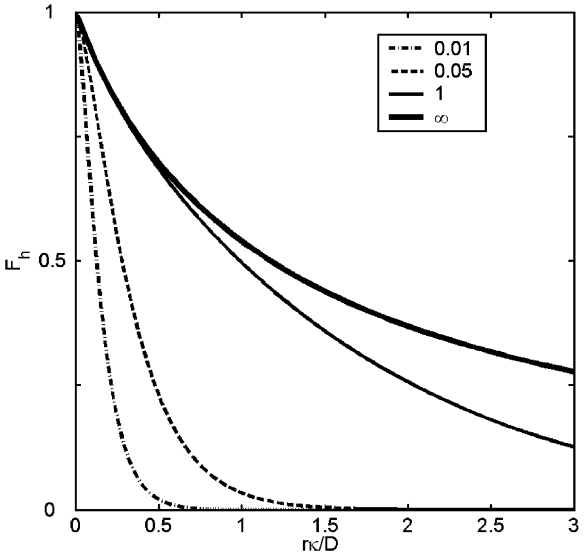

Fig. 4. Fraction of trajectories trapped at distances larger than r, evaluated at several values of dimensionless parameter $h\kappa/D$. The legend shows the curves corresponding to the different parameter values.

intermediate regime, one can use the general solution in Eq. (5).

Our analysis can be extended to include the effects of additional processes. For example, in cell culture experiments, the dynamics of secreted ligands is frequently affected by the addition of antibodies that reversibly bind the ligand, making it temporarily unavailable for receptor binding [21]. This means that a ligand randomly jumps between the reactive and non-reactive states. Ligand receptor binding is just one of the receptor-mediated steps that control the spatial range of secreted ligands. In the future, it will be important to combine our description of extracellular transport and receptor binding, with kinetic models of ligand endocytosis, recycling and degradation, to develop a comprehensive description of receptor-mediated transport in tissues [5–7].

Appendix A: Probability density for the ligand lifetime

The probability density for the ligand lifetime can be expressed in terms of the propagator $g(z, t)$

$$\varphi_h(t) = \kappa g(0, t) \tag{A1}$$

The propagator, or the Green's function, is the probability density to find a ligand at distance z from the partially absorbing boundary at time t, on condition that it started from this boundary at $t=0$. The propagator satisfies the diffusion equation

$$\frac{\partial g}{\partial t} = D\frac{\partial^2 g}{\partial z^2} \tag{A2}$$

with the initial and boundary conditions of the form

$$g(z,0) = \delta(z), \quad \left.\frac{\partial g}{\partial z}\right|_{z=0} = \frac{\kappa}{D} g(0,t), \quad \left.\frac{\partial g}{\partial z}\right|_{Z=h} = 0 \tag{A3}$$

Using the eigenfunction expansion of the propagator one can write $\varphi(t)$ as

$$\varphi_h(t) = \kappa \sum_{n=1}^{\infty} [\psi_n(0)]^2 \exp(-k_n^2 D t) \tag{A4}$$

where $\psi_n(z)$ are the corresponding eigenfunctions and k_n are positive roots of Eq. (4). Solving the eigenvalue problem one derives Eq. (4) that determines k_n. Simultaneously one finds

$$[\psi_n(0)]^2 = \frac{2D^2 k_n^2}{D\kappa + h(\kappa^2 + D^2 k_n^2)} \tag{A5}$$

Substituting this into Eq. (A4) one arrives at the expression for $\varphi_h(t)$ in Eq. (3).

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